A new concept for isotope ratio monitoring LC/MS

A Wide Range of Applications

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Overview

- Introduction in *Isotope Ratio Monitoring* - LC/MS (*irm* - LC/MS)
  - ***Technology***
  - ***Operating Modes***

- New Applications by *irm* - LC/MS
  - ***Authenticity Control***
    - Detection of adulteration by sugars in honey.
  - ***Determination of Origin***
    - Differentiation of analgesic drugs.
  - ***Molecular Biology***
    - Carbon isotopic characterization of rRNA.
  - ***Biogeochemistry***
    - Plant metabolism study of organic acids.
  - ***Forensic Chemistry***
    - Analysis of aspartic acid in cadaver blood samples.

Why irm – LC/MS ?

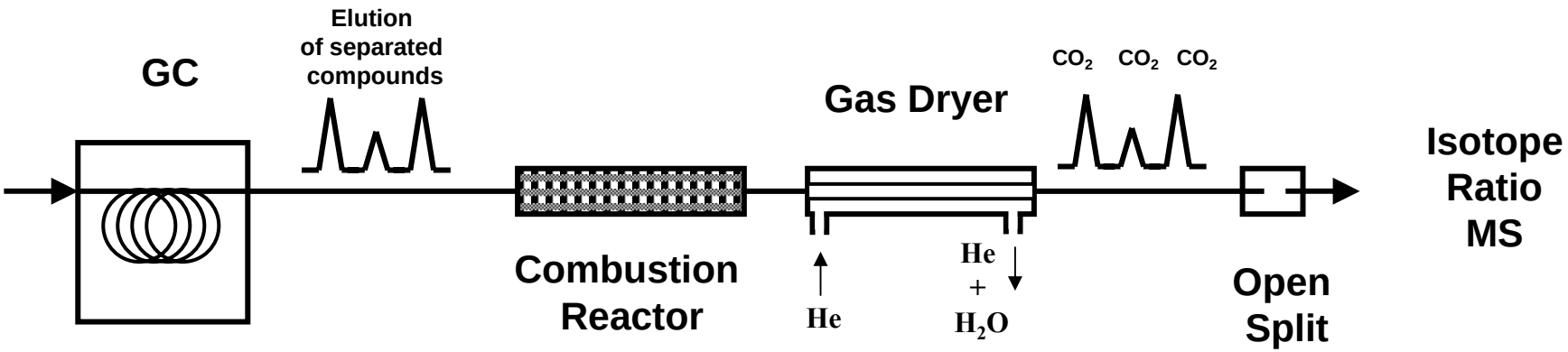
- $\delta^{13}C$ analysis of individual compounds with:

  – *High molecular weight*
  – *High polarity*
  – *Thermal instability*
  – *Low vapour pressure*

  – *Less sample preparation*
  – *No derivatization*
  – *No isotope dilution*
  – *Less risk of fractionation*

Comparison between irm GC/MS and irm LC/MS

- irm – GC/MS

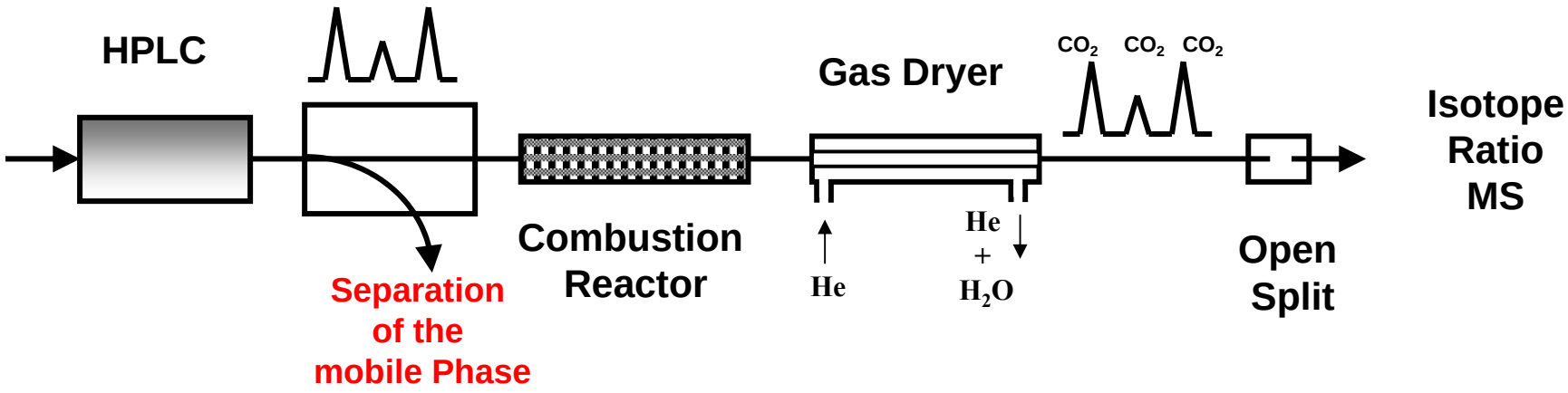


- Helium as carrier for
  - *Separation of compounds*
  - *Transfer to the IRMS*
- Helium has
  - *No impact on combustion*
  - *No effects in the IRMS*

Dry combustion (oxidation) in the He phase

Comparison between irm GC/MS and irm LC/MS

- irm−LC/MS (first strategy)

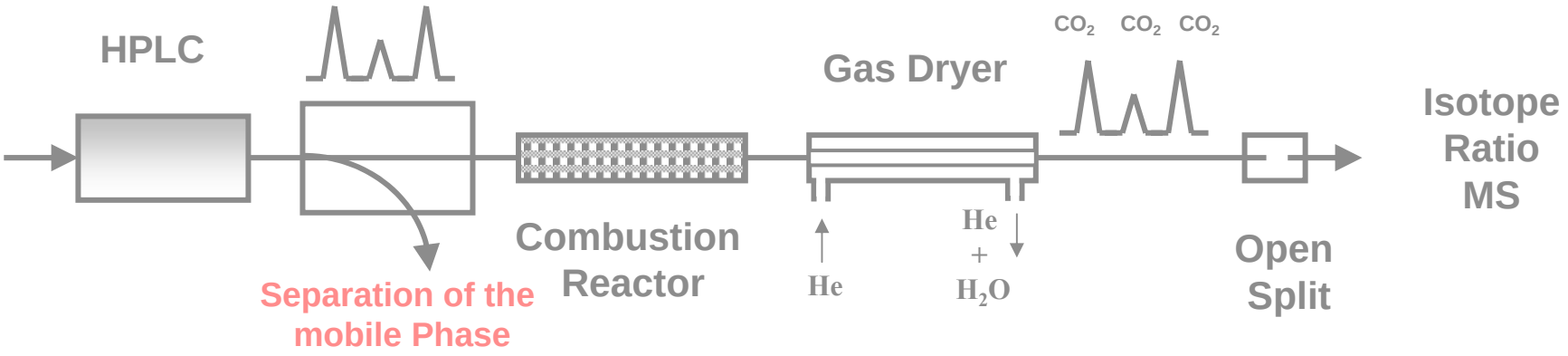


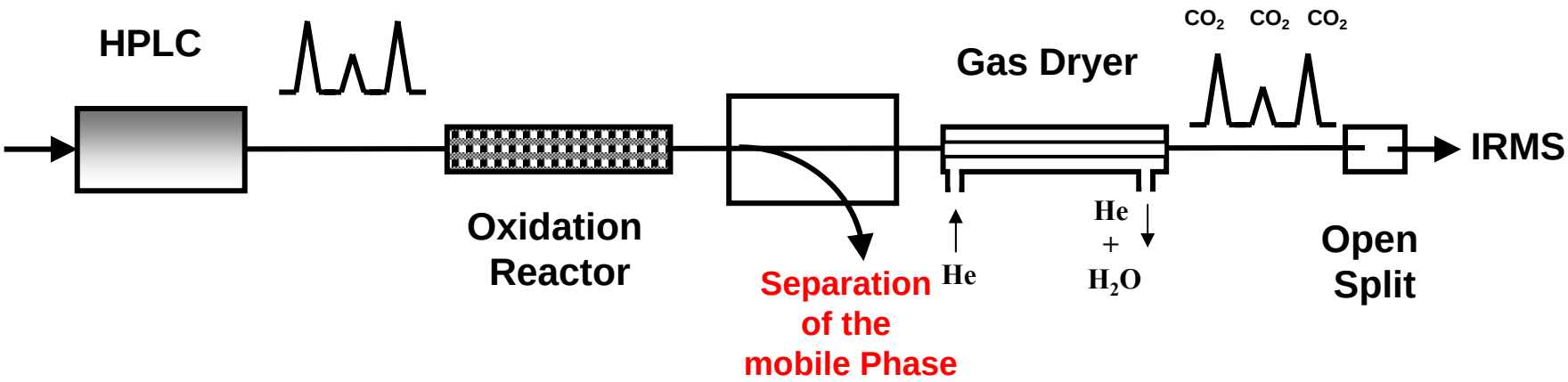
- Solvents as carrier for
  - *Separation of compounds*
- Solvents are
  - *Oxidized*
  - *Hazardous to IRMS*
- No solvents to reactor or IRMS

First approaches (’91, ’93)

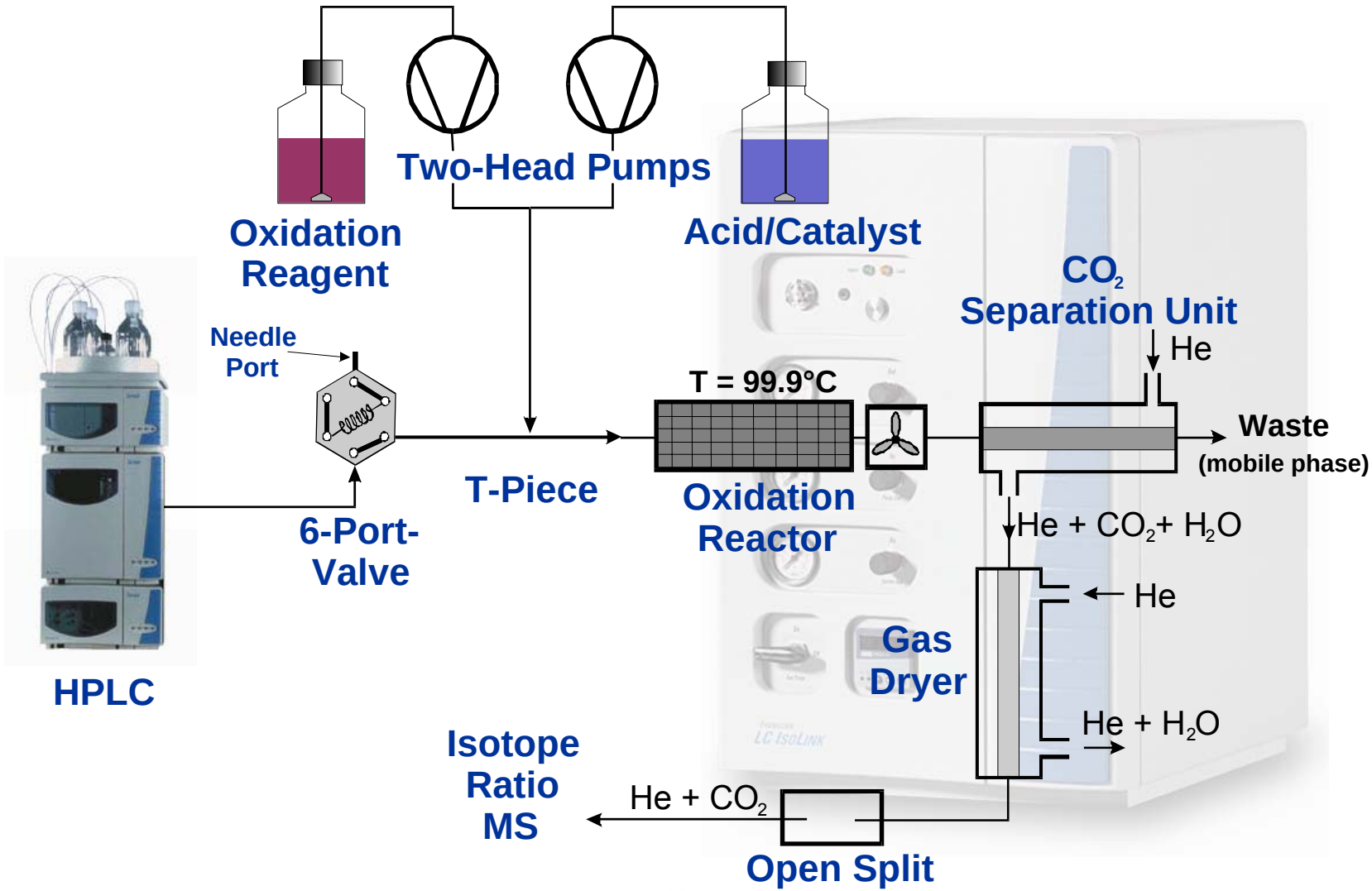
- *“Moving Wire” – Drying system (difficult to use)*
- *“Particle Beam” Separation (low sensitivity, fractionation)*

A New Strategy



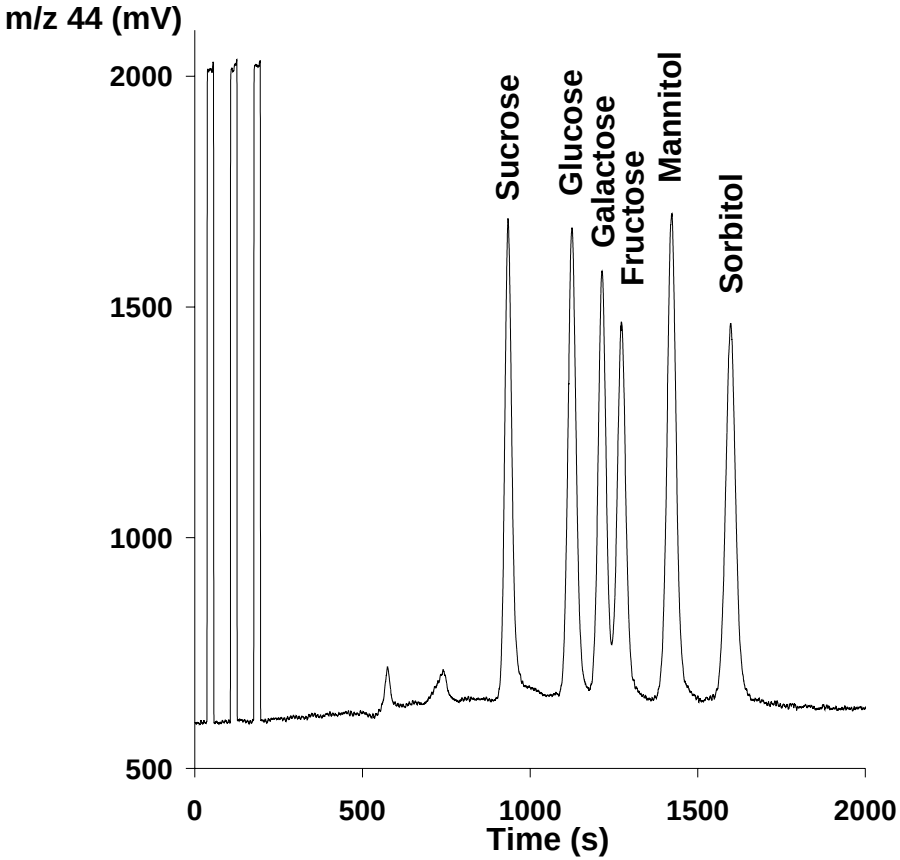


Scheme of the LC IsoLink Interface



HPLC Resolution

HPLC separation of carbohydrates

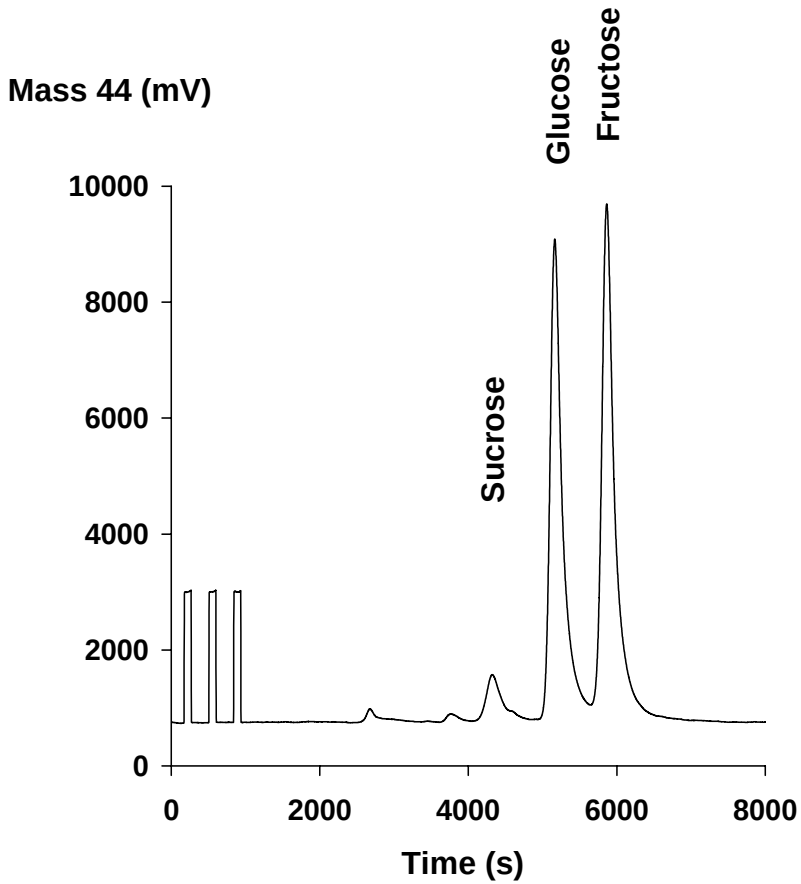


HPLC resolution is maintained

- Parameters:
  - *HPLC flow:*
    - 300 µl/min
  - *Oxidation reagent:*
    - 60 µl/min $(NH_4)_2S_2O_8$, 100g/l
  - *Column:*
    - 700 CH Carbohydrate Column, 90 °C
  - *Reactor:*
    - 99.9 °C
  - *CO_2 Exchanger:*
    - 1 ml/min He flow

Authenticity Control of Honey

Investigation of the adulteration of honey analyzing glucose, sucrose, fructose

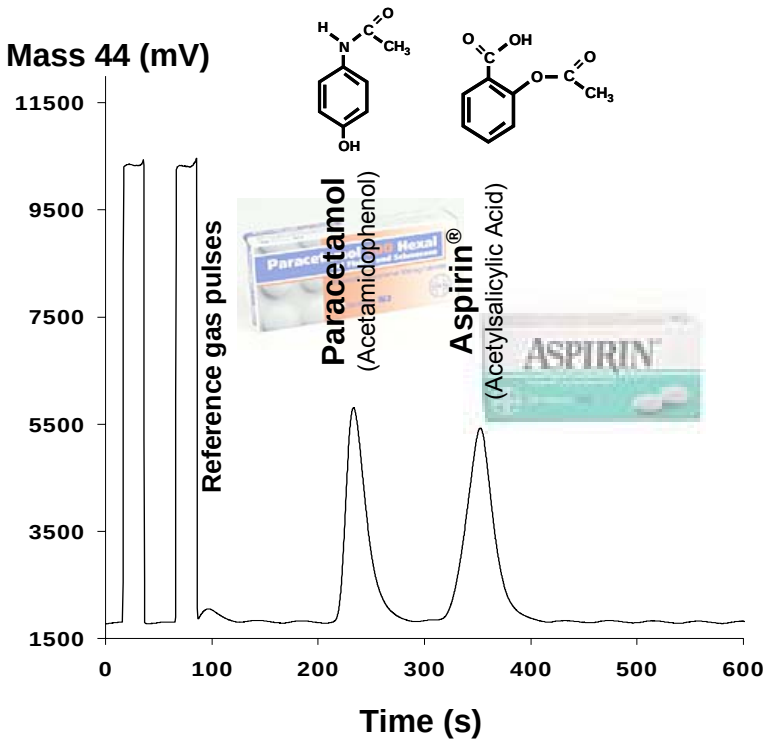


Honey	Glucose $\delta^{13}C‰$	Fructose $\delta^{13}C‰$	Area Fru/Glu	
A	-27.9	-27.8	1.13	pure
B	-25.1	**-26.4**	2.17	**adulterated**
C	-26.5	-26.5	1.35	pure
D	-26.1	-26.0	**4.53**	**adulterated**
E	-11.2	**-13.9**	0.65	**adulterated**

- Absolute $\delta^{13}C$ value
- $\delta^{13}C$ difference, Glu – Fru
- Ratio of area, Fru / Glu

Source Differentiation of Drugs

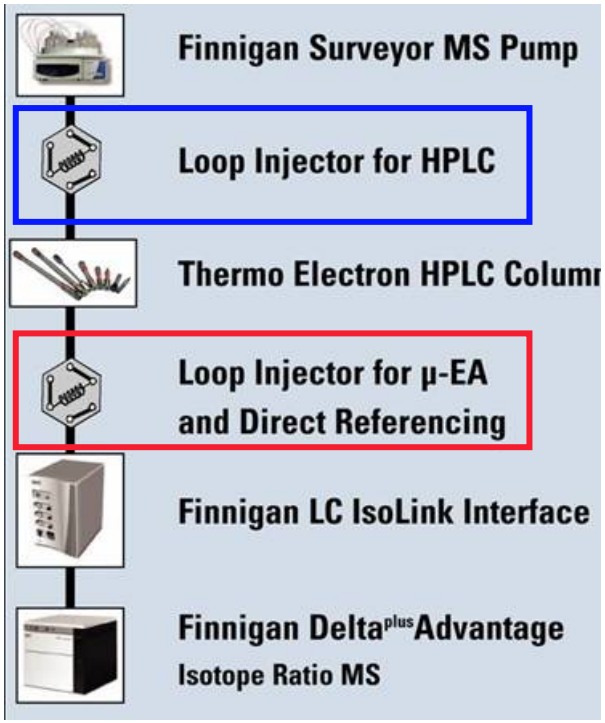
Determination of different analgesic compounds.
$\delta^{13}C$ of Paracetamol (Acetamidophenol) and Aspirin® (Acetylsalicylic Acid; ASA).



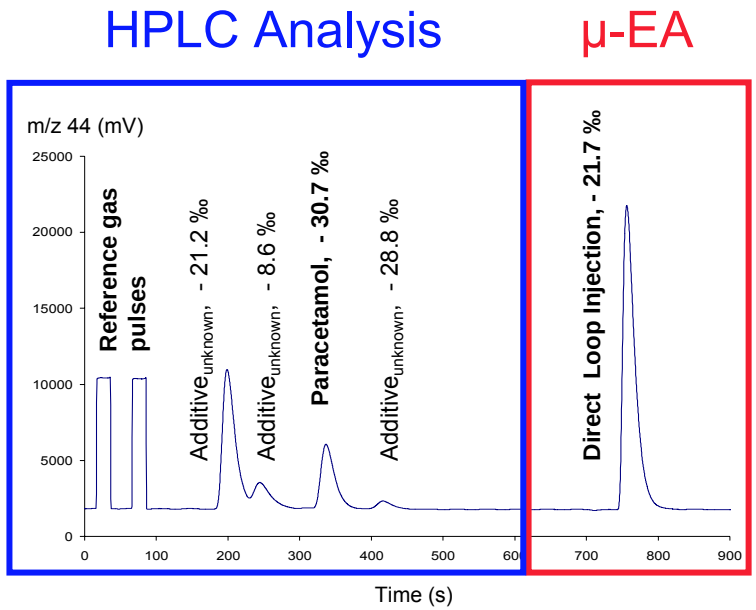
Tablet Type	$\delta^{13}C$ (‰) Paracetamol	ASA	μ-EA
A Country1	-	**-34.2**	**-33.4**
A Country2	-	**-34.2**	**-33.5**
B	-	-29.1	-27.6
C	-	-27.2	-26.6
D_1	-	-26.8	-26.7
E	-	-27.7	-27.6
F	-32.3	-32.6	-31.7
D_2	-28.7	-33.8	-31.3
G	-29.2	-32.7	-29.7

- Tablet type A has the same origin
- 4 sources of ASA
- Producer D use different ASA sources

µ-EA – Direct Injecton



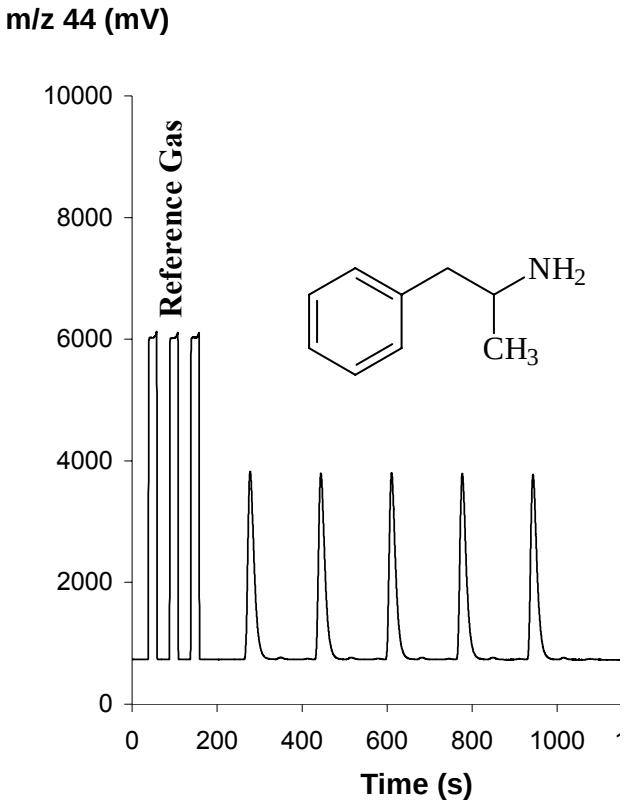
- Fast analysis of all water soluble compounds



Analysis of a tablet followed by direct loop injection (µ-EA). Loop size of the HPLC injector was 5 uL, the loop size of the µ-EA injector was 10 uL, which results in two-fold response of the µ-EA peak

µ-EA – Reproducibility

Bulk Injection of Amphetamine

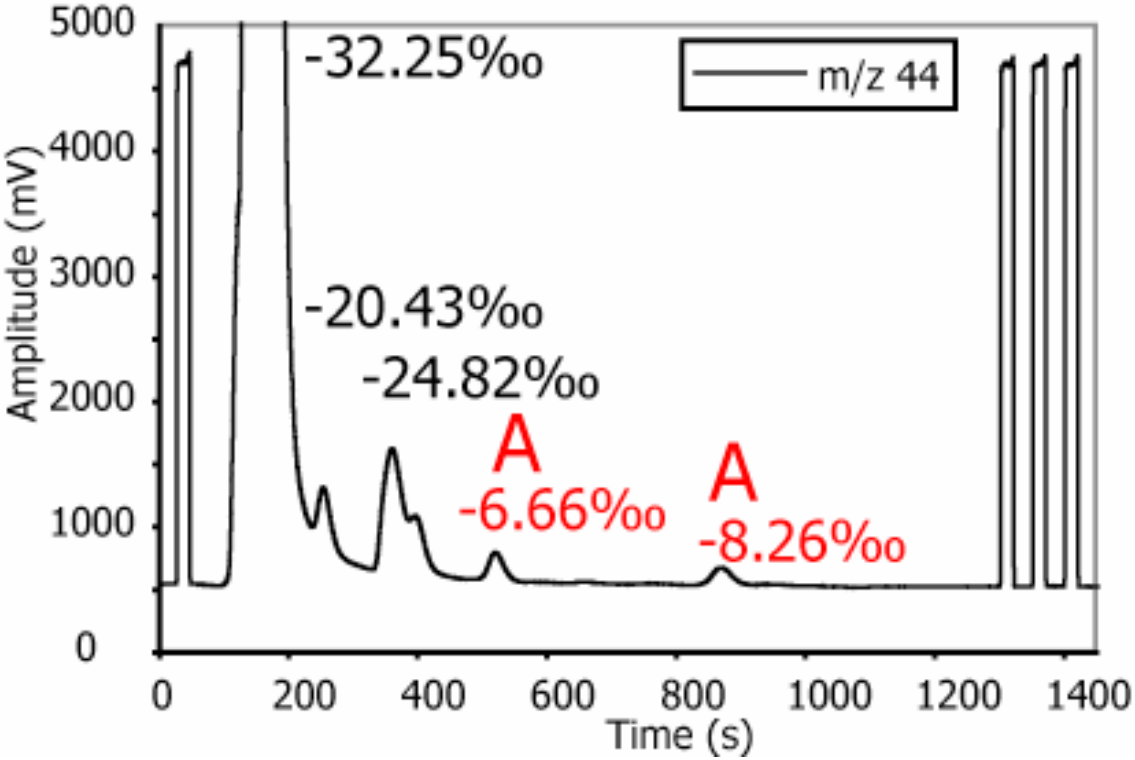


e.g., 5 x bulk injections of 218 ng amphetamine

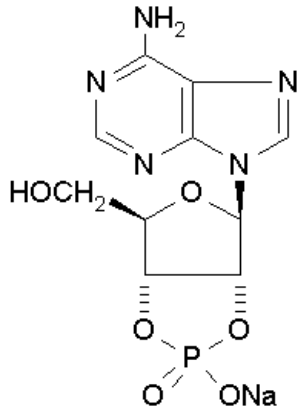
Sample	Amount Amphetamine (ng)	Amount Carbon (ng)	$\delta^{13}C$ (‰)	S.D. (‰)	n
#1	218	174	-28.70	0.04	5
	452	362	-28.76	0.04	5
	698	558	-28.83	0.03	5
	944	756	-28.87	0.03	5
	1161	929	-28.76	0.03	5
	1359	1088	-28.67	0.02	5
	2280	1824	-28.70	0.02	5
Mean			**-28.76**	**0.03**	
#2	732	586	**-32.23**	**0.04**	5
#3	438	350	**-31.58**	**0.04**	5
#4	781	625	**-27.89**	**0.03**	5

Reliable reproducibility of the $\delta^{13}C$ values

irm-LC/MS of NaOH-hydrolyzed *E. coli* RNA



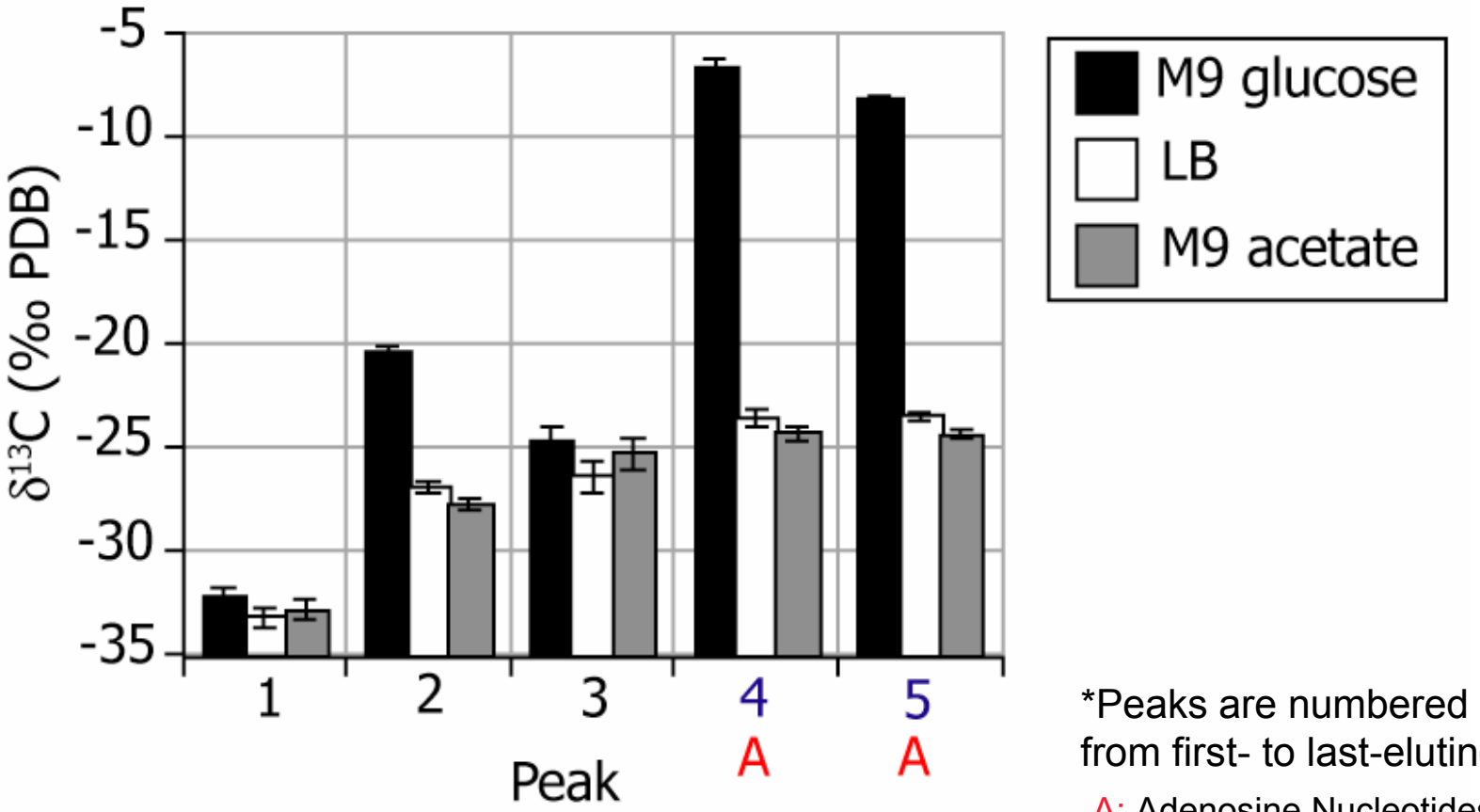
Linkage of microbiological species identity with carbon source utilization by carbon isotopic characterization of rRNA.



- Cell growth and Isolation: *RNA was extracted from overnight cultures of E. coli grown on M9 minimal salts with glucose as sole carbon source Medium.*
- Hydrolyse: *0.2 N NaOH for 15 min at 50 °C*

A: Adenosine Nucleotides

Carbon Isotopic Composition of Individual Peaks from NaOH-hydrolyzed E. coli RNA

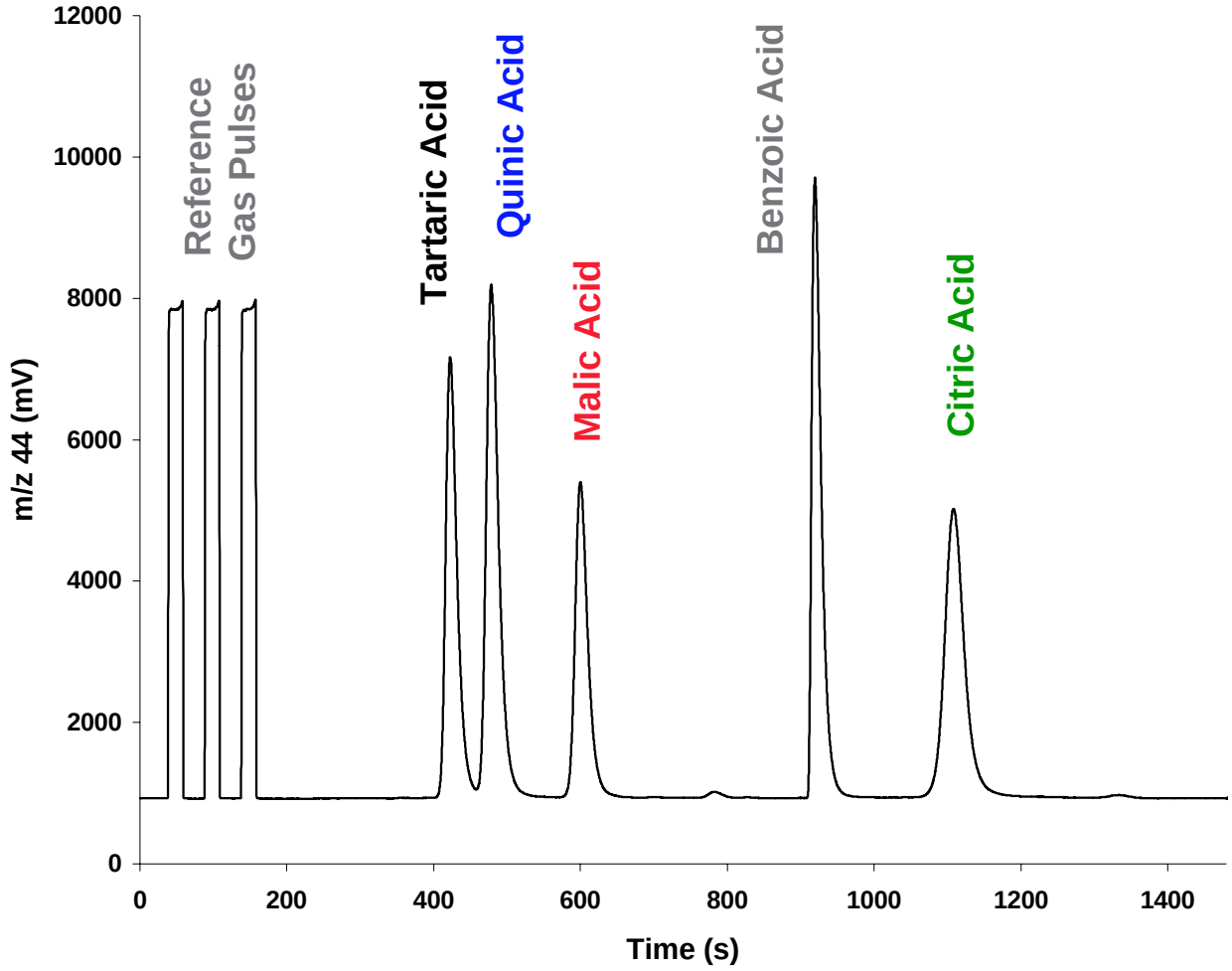


*Peaks are numbered from first- to last-eluting

A: Adenosine Nucleotides

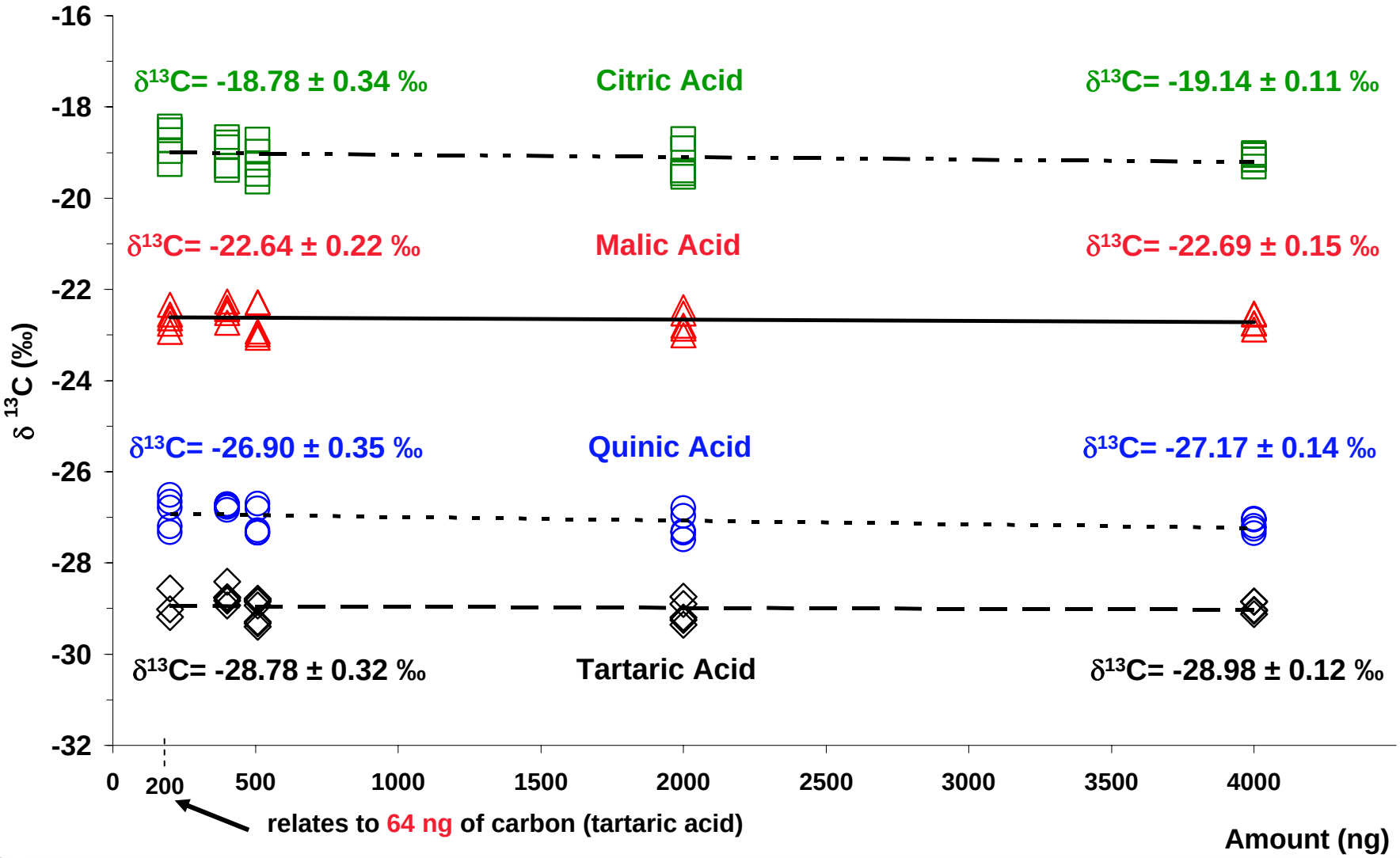
⇒ **The carbon isotopic composition of RNA closely reflects that of the growth substrate.**

$\delta^{13}C$ Analysis of Fruit Juice Organic Acids



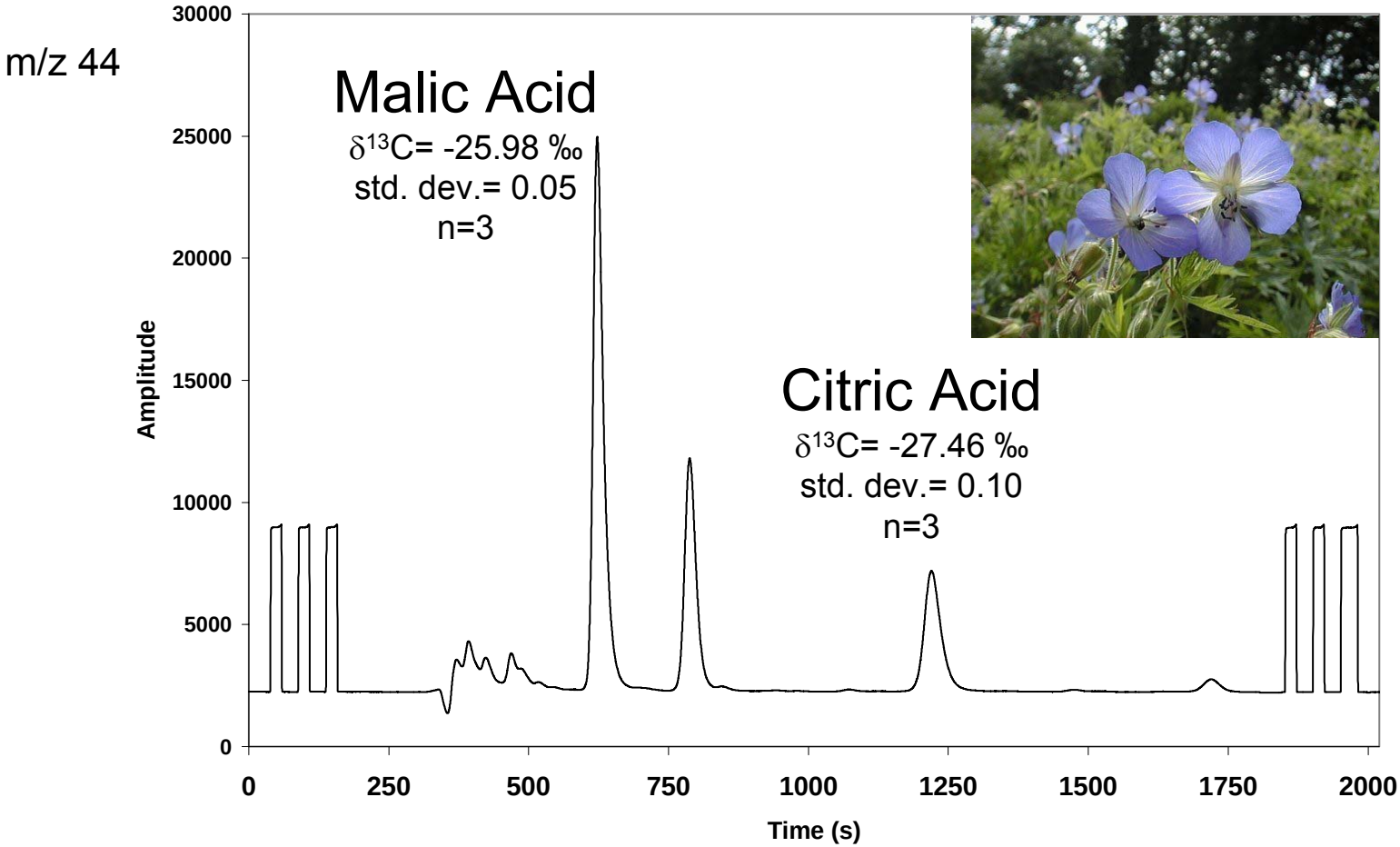
- **HPLC Column:**
  Allure Organic Acids,
  300 mm x 4.6 mm, 5 µm
- **Flow:**
  500 µl/min
- **Mobile Phase:**
  100 mM KH_2PO_4, pH 3

$\delta^{13}C$ Analysis of Organic Acids

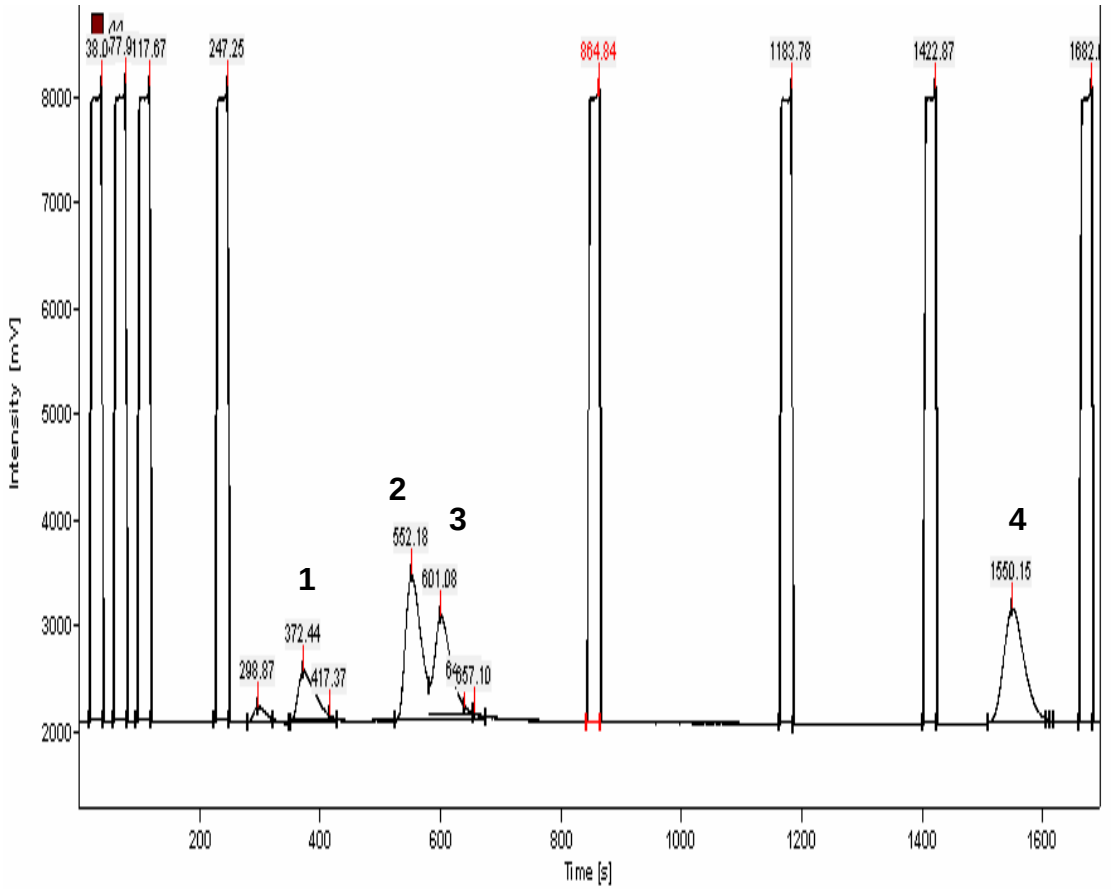


irm-LC/MS: $\delta^{13}C$ Analysis of an Extract of *Geranium pratense* (May 2004)

Plant metabolism study of organic acids



Analysis of Volatile Fatty Acids by irm-LC/MS



1. **Formate**
2. **Lactate**
3. **Acetate**
4. **Propionate**

Pump 1: 200 mg/l $(NH_4)_2S_2O_8$, 50µl/min

Pump 2: 2M H_3PO_4, no $AgNO_3$-catalyst, 50µl/min

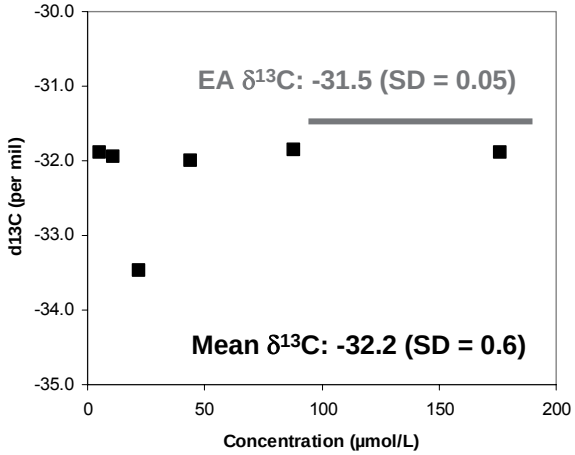
Reactor: 99.9°C

Data: Prof. Hinrichs Univ. Bremen

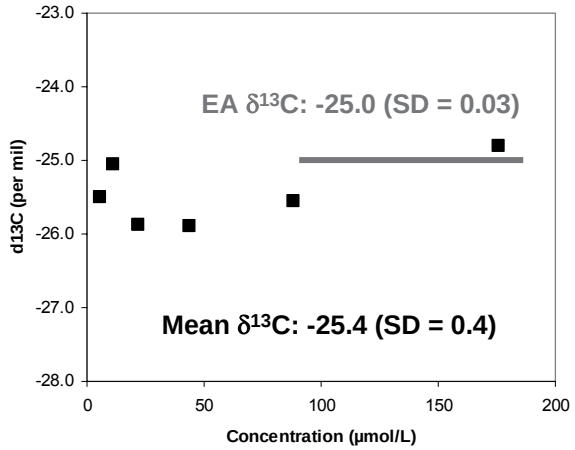
Linearity of the IRMS - Signal

Amounts injected : 8.8 nmol – 0.28 nmol

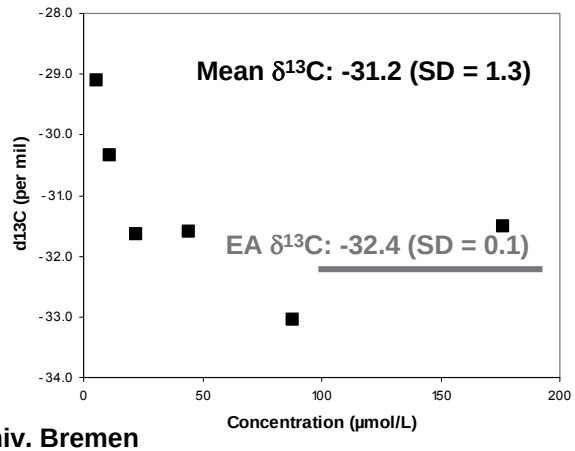
Formate



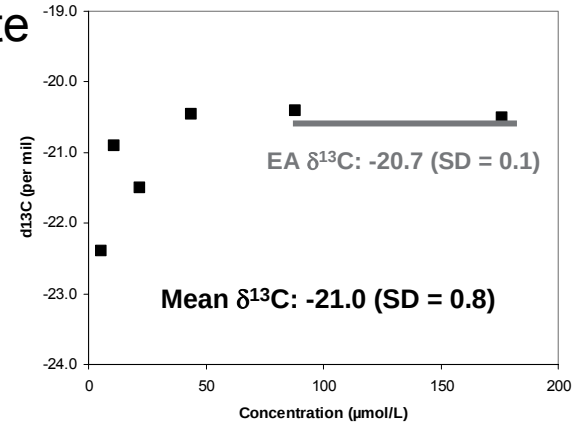
Lactate



Acetate

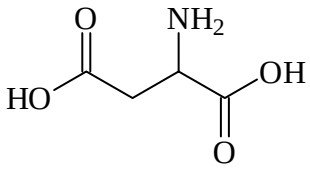


Propionate

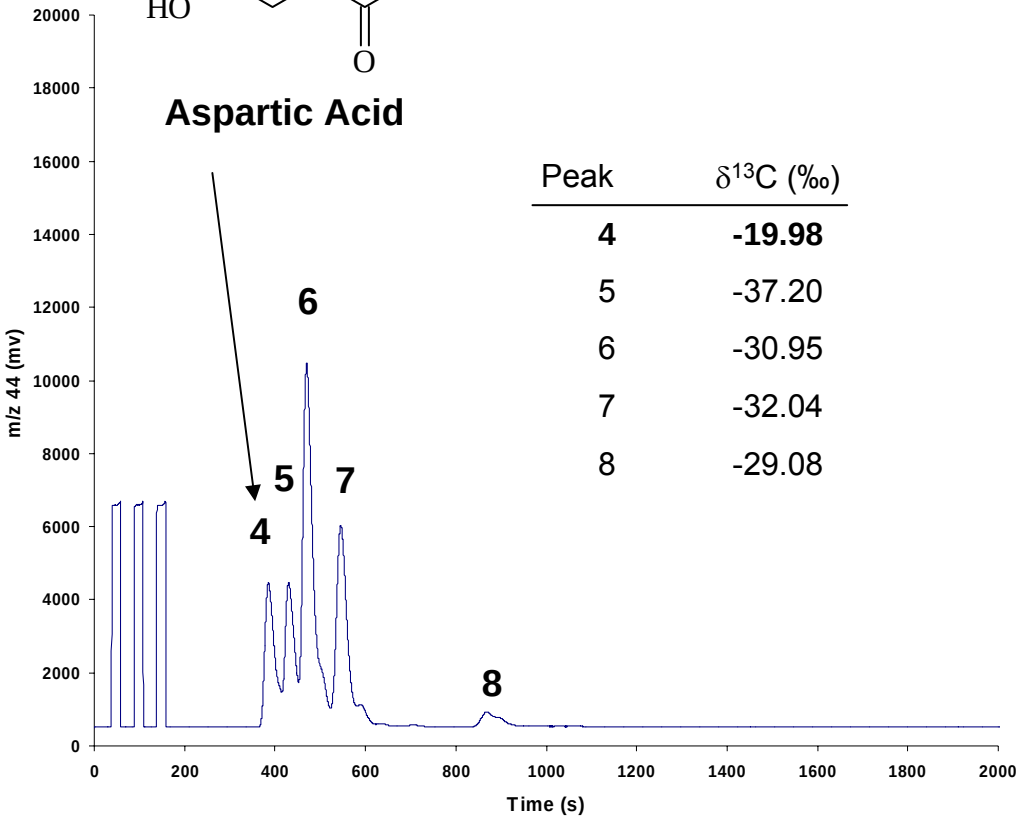


Data: Prof. Hinrichs Univ. Bremen

$\delta^{13}C$ Analysis of Aspartic Acid in Cadaver Blood



Aspartic Acid



Peak	$\delta^{13}C$ (‰)
4	**-19.98**
5	-37.20
6	-30.95
7	-32.04
8	-29.08

Aspartic Acid

Sample	$\delta^{13}C$ (‰)	Std. Dev.
A	-32.12	0.08
B	-32.66	0.11
C	-16.18	0.03
D	-18.76	0.14
E	-19.98	0.06
F	-16.62	0.12
Standard		
G	-24.79	0.05
H	-26.19	0.11

- Mobile Phase: 10mM $NaH_2PO_4*H_2O$, pH 4.7, Flow: 300 µl/min
- HPLC Column: Nova-Pack® C18, 60 Å (4 µm, 3.9 mm x 300 mm).

Summary

- *irm*-LC/MS opens a wide range of interesting applications.
- Macromolecules, non-volatile components and components which tend to decompose are directly accessible for precise isotopic analysis.

Acknowledgements

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